


Study Summary Review Outline: Clayton Myers, IB


3-10-09

Decision #: 399095

DP #: 357273

MRID: 475185-13

Title: Efficacy of a Combination of Etofenprox and PBO (RF2042B) as a Topical Spot-On (sic) for Dogs Experimentally Infected (sic) with Adult *Ixodes scapularis* Ticks and Three Species of Adult Mosquitoes (*Aedes aegypti*, *Anopheles quadrimaculatus*, and *Culex quinquefasciatus*).

Purpose/Objectives:

The study was designed to evaluate the efficacy of a topical spot-on RF2042B application on dogs against:

Black-legged tick (BLT) (deer tick)—residual adulticidal activity

Mosquito (*Aedes aegypti*)—residual adulticidal activity and blood feeding

Mosquito (*Anopheles quadrimaculatus*)—residual adulticidal activity and blood feeding

Mosquito (*Culex quinquefasciatus*)—residual adulticidal activity and blood feeding

Materials and Methods:

Animals: 16 healthy dogs, 11 males, 5 females from BerTek, Inc. colony, of varying ages and weights (1-7.5 years, and 12.5-30.2 lbs). Dogs were not treated with any insecticides within four weeks of Day 0. Bertek standard housing and feeding protocol was used.. These 16 dogs were chosen from a group of 20 (12 males, 8 females) based on pre-treatment qualification with flea retention.

Test parasites: Cat fleas from an in-house colony. BLT from Oklahoma State University, Mosquitoes from Benzon Research, Inc.

Test insecticide treatments are not described as fully as in 475185-11

Design:

2 treatments: a control group (1), and an RF2042B insecticide treated group (2)

Replicates: 6 dogs in control (group 1), 10 dogs in insecticide treated (group 2)

Randomization: Dogs were ranked by weight and then assigned to the two groups randomly (per a table in appendix 3, not included in packet—this was discussed under section 9.0 “Deviations.”).

Dosages: 1.5 ml for dogs weighing up to 15 lbs, 3.0 mL for dogs weighing from 16 to 30 lbs. Material administered to each dog along the dorsal line in approximately 3 equal spots—one between shoulders, one in the middle of the back, one at the base of the tail. There was one application to all dogs in group 2, with no re-treatments.

Infestations: Ticks were infested *in vitro* onto hair collected on Days 7, 14, 21, 28, 35, 42, and 50 post-treatment. Approximately 1 gram of fur was clipped from approximately the same body site of each study animal and placed in containers with the dog ID and time/date of sampling. Ten ticks (pre-counted in vials) were placed in each container of hair. Containers were stored in a growth chamber. Starved mosquitoes (12 h without sugar solution) were placed in crates within tents. For infestation, approximately 25-50 unfed, unsexed mosquitoes of each species were placed within the tents by aspiration (simultaneously). Once all tents were infested, room lights were turned off until recollection began. Gloves and aspirators were changed between treatments to avoid any cross contamination of insecticide residues.

Data collection:

Ticks: Tick counts were made on days 8, 9 (via visual counts on the same hair) and on days 10 (still same initial hair), 17, 24, 31, 38, 45, and 53 (via a counting tray and disposal of hair and ticks), approximately 24 hours after initial infestation and 72 hours after each weekly re-infestation.

Mosquitoes: Collections were conducted 2 hours after each tent infestation on days 0, 14, 21, 28, 35, and 42 via vacuum aspiration. Mosquitoes were placed in separate labeled vials and scored as alive or dead. For sorting, mosquitoes were sorted by species and sexed, and all females were squashed to determine if feeding occurred.

Statistics: Only descriptive statistics are given. Geometric means were calculated for each group and then % control was calculated by comparing treatment means to the control. $\% \text{ control} = ([\text{GM ctrl} - \text{GM trt}] / \text{GM ctrl}) * 100$

Study Summary of the Results:

Treatment provided $\geq 98\%$ *in vitro* control of BLT through day 45

For *Ae. aegypti*, mortality was $\geq 95\%$ through day 35 and feeding reduction remained $\geq 94\%$ through day 42.

For *Cx. quinquefasciatus*, mortality control was $\geq 94\%$ only on day zero, but feeding reduction remained $\geq 95\%$ through day 28.

For *An. quadrimaculatus*, mortality was $\geq 98\%$ through day 42, and feeding reduction remained $\geq 83\%$ through day 35.

Entomologist's Observations/Discussion

Treatment provided $\geq 98\%$ in vitro control of BLT through day 45, before falling off to 69% control at day 53.

For *Ae. aegypti*, mortality was $\geq 95\%$ through day 35 and feeding reduction remained $>94\%$ through day 42.

For *Cx. quinquefasciatus*, there was no knockdown efficacy past day zero, but feeding reduction remained $\geq 95\%$ through day 28.

For *A. quadrimaculatus*, mortality was $\geq 98\%$ through day 42, and feeding reduction remained $>83\%$ through day 35.

Observations/Discussion:

1. The qualification of dogs was for flea acceptance, not tick acceptance. Although the tick assay was only *in vitro*, it would have been more appropriate to test acceptance of ticks than to test with fleas, since flea control efficacy was not being evaluated in this study.
2. BLT results are difficult to extrapolate to specific claims because there was no field exposure of dogs, and it's an *in vitro* study."
3. For hair sampling for tick studies, there is no specification of what area the hair was taken from; only that it was 'approximately the same body site' of each dog. There is no indication if sites were changed or randomized. They could have taken hair directly from the site of insecticide treatment and biased the sample.
4. On tick data, the methods indicates that ten ticks were placed on each gram of hair (prepackaged in counted groups of ten) and then mortality was assessed. But if ten were placed, then the data should work out to even percentages (i.e., 10%, 20%, 30%, etc.) for % alive and for mortality for each dog. Either a number different than ten was used, or something was worded wrongly in the methodology, because % alive and mortality numbers are not often even multiples of ten. The registrant needs to explain this discrepancy.
5. "Approximately 25-50" mosquitoes of each species were placed in each cage." There is tremendous variability, and what is the assurance that this was randomized? If, for example, only 25 mosquitoes were placed in the treatment 2 tents, and 50 mosquitoes were placed in the treatment 1 tents, then calculation and comparison of GM's would lead to 50% "mosquito control" even if there was no treatment effect whatsoever. There was also no description given in this study of what the sex ratios are—this is important, because male mosquitoes are not blood feeders and efficacy against males would not be relevant to protection of the dog

from blood feeding. We need to see the raw data on this portion of the study. How many female mosquitoes were evaluated for each species to calculate the % blood feeding and mortality results?

6. OPPTS 810.3300 guidance indicates that there should be 95% knockdown efficacy on mosquitoes to make a mosquito claim. This was not the case for *Culex*, but the data does indicate that blood feeding is reduced there, albeit only through day 28.
7. The label claims “kills and repels” mosquitoes. Based on lack of blood feeding the data show that mosquitoes were “repelled”,
8. The label claims are acceptable except as noted below:

Effective on Indoor and Outdoor dogs—Not acceptable, since dogs were not exposed to outdoor conditions in any of the studies.

Each tube [applicator] kills fleas & ticks for 3 to 4 weeks and kills and repels mosquitoes for 3-4 weeks—Not acceptable. Kills fleas and ticks for up to 3 weeks. Mosquito efficacy varied by species. Claims of 3 to 4 weeks are acceptable for killing *Aedes aegypti*, and *Anopheles quadrimaculatus*, but not *Culex quinquefasciatus*. Claims of repellence are acceptable

Kills and repels mosquitoes (a major carrier of canine heartworm)—Acceptable if killing is qualified by species, since it doesn't kill *Culex*.

RF2042 [CDSO] prevents flea eggs from developing into adult fleas [more than 10 weeks] for up to [75 days][2.5 months]. RF2042 [CDSO] also kills fleas and ticks for 3 to 4 weeks and kills and repels mosquitoes for 3 to 4 weeks—Not acceptable. Kills fleas and ticks for up to 3 weeks is acceptable, but mosquito efficacy varied by species. Kill claims of 3 to 4 weeks are acceptable for *Aedes aegypti*, and *Anopheles quadrimaculatus*, but not *Culex quinquefasciatus*. Repellence claims are acceptable.

[RF2042 [CDSO] is water resistant and remains efficacious following exposure to rainfall or swimming.]—Unacceptable. Water resistance/rainfastness was not demonstrated in this study.